CLAIMS

We claim:

1. A culture medium for culturing a strain of *Corynebacterium diphtheriae* to produce a level of diphtheria toxin or an analog thereof wherein the medium is substantiallty free of animal-derived products and comprises
   a. water;
   b. a carbohydrate source and a nitrogen source;
   c. a number of free amino acids in an initial concentration wherein the initial concentration of each free amino acid is not limiting for the level of production of the diphtheria toxin or the analog thereof.
2. The culture medium of claim 1 comprising all naturally occurring amino acids.
3. The culture medium of claim1 wherein the carbohydrate source comprises maltose.
4. The culture medium of claim1 substantially free of glucose.
5. The medium of claim 1 wherein the nitrogen source comprises yeast extract.
6. The culture medium of claim 1 or 2 or 3 or 4 or 5 wherein the medium is devoid of animal-derived products.
7. A medium for *Corynebacterium diphtheriae* comprising:
   a carbohydrate source and a nitrogen source and an additive system that comprises at least four free amino acids being each in an amount sufficient to promote a level of diphtheria toxin or an analog thereof production by *Corynebacterium diphtheriae* wherein the medium is substantiallty free of animal-derived products.
8. The culture medium of claim 7 comprising all naturally occurring amino acids
9. The medium of claim 7, wherein the carbohydrate source is maltose.
10. The medium of claim 7, wherein the nitrogen source is yeast extract.
11. The medium of claim 7 or 8 or 9 wherein amino acid concentrations are in the range from about to 0.5 grams to about 1gram per litre of the medium.
12. The culture medium of claim 7 or 8 or 9 or 10 or 11 wherein the medium is devoid of animal-derived products.
13. A method for the production of diphtheria toxin or an analog thereof comprising the steps of:
    culturing a strain of *C. diphtheriae* in a culture medium under conditions that allow

production of diphtheria toxin, wherein the culture medium is substanitailly free of animal-derived products and comprises comprises water;

a. a carbohydrate source and a nitrogen source;

b. a number of free amino acids in an initial concentration wherein the initial concentration of each free amino acid is not limiting for the level of production of the diphtheria toxin or the analog thereof.

14. The method of claim 13, wherein the culture medium comprises all naturally occurring amino acids.

15. The method of claim 13, wherein the carbohydrate source comprises maltose.

16. The method of claim 13, wherein the culture medium is substantially free of glucose.

17. The method of claim 13, wherein the nitrogen source comprises yeast extract.

18. The method of claim 13 wherein the *C. diphtheriae* strain is grown until stationary phase.

19. The method of claim 13 wherein a production of at least 100 Lf/mL of diphtheria toxin or analog thereof is obtained.

20. The method of claim 10 further comprising a step of recovering the diphtheria toxin or analog thereof to provide a recovered diphtheria toxin or analog thereof.

21. The method of claim 17 further comprising a step of purifying the recovered diphtheria toxin or analog thereof to provide a purified diphtheria toxin or analog thereof.

22. The method of claim 17 or 18 further comprising a step of detoxifying the recovered or purified diphtheria toxin or analog thereof to provide a diphtheria toxoid or analog thereof.

23. The method of claim 19 further comprising formulating the diphtheria toxoid or analog thereof as a vaccine for immunizing a host agaisnt diseaase caused by infection by *C. diphtheriae.*

24. The method of any one of claism 13-23 wherein the medium is devoid of animal-derived products.

25. A method of immunizing a host against disease caused by infection by *C. diphtheriae* comprising administering the vaccine of claim 23 to the host.

26. The use of the vaccine of claim 23 for immunizing a host agaisnt diseaase caused by infection by *C. diphtheriae.*

27. The use of the diphtheria toxoid or analog thereof of claim 22 in the prepration of a medicament for immunizing a host agaisnt disease caused by infection by *C. diphtheriae.*

28. A composition comprising a *C. diphtheriae* strain and a culture medium for producing diphtheria toxin or analog thereof wherein the medium is substantiallty free of animal-derived products and comprises wherein the culture medium is substanitailly free of animal-derived products and comprises comprises
    a. water;
    b. a carbohydrate source and a nitrogen source;
    c. a number of free amino acids in an initial concentration wherein the initial concentration of each free amino acid is not limiting for the level of production of the diphtheria toxin or the analog thereof.
29. The composition of method of claim 24, wherein the culture medium comprises all naturally occurring amino acids.
30. The composition of method of claim 24, wherein the carbohydrate source comprises maltose.
31. The composition of claim 24, wherein the culture medium is substantially free of glucose.
32. The composition of method of claim 24, wherein the nitrogen source comprises yeast extract.
33. The culture medium of claim 21 or 22 or 22 or 23 or 24 or 25 wherein the medium is devoid of animal-derived products.
34. A method for producing diphtheria toxin or an analog thereof comprising growing a culture of *Corynebacterium diphtheriae* in a medium and providing at least one selected amino acid to the culture to prevent concentrations of the selected amino acids being limiting for toxin production wherein the medium is substantiallty free of animal-derived products.
35. The method of claim 1 wherein the medium further comprises a yeast extract.
36. The method of claim 4 wherein the yeast extract is present at a concentration of about 3g/L. concentration of amino acids.
37. In a culturing method of *Corynebacterium diphtheriae* in a medium containing amino acids for producing a level of production of diphtheria toxin or an analog thereof and in which at least one selected amino acid is depleted during the culturing and limits the level of production of the diphtheria toxin or the analog thereof, the improvement comprising an exogenous addition of an additional amount of the at least one selected amino acid

during said culturing and wherein the at least one selected amino acid is not limiting for the level of production of the diphtheria toxin or the analog thereof.

38. The method of claim 33 wherein the at least one selected amino acid is selected from the group consisting of Glu, Asn, Ser, His, Gly, Thr, Met, Trp, and Isoleucine.

39. The method of claim 33, wherein the medium comprises a yeast extract.

40. The method of claim 35 wherein the yeast extract is present at a concentration of about 3g/L.